In the outstanding Official Action, the specification was objected to for allegedly containing sequences which lacked sequence identification numbers. It is believed that the present amendment obviates this objection.

The specification has been amended so as to provide sequence identification numbers for the sequences found on pages 31 to 34. Thus, it is believed that the present application now complies with the requirements for patent applications containing nucleotide and/or amino acid sequences.

The outstanding Official Action also objected to pages 34 and 35 of the present specification for referring to trademarks.

It is believed that the specification has been amended so that the trademarks are capitalized wherever they appear in the disclosure. Moreover, the specification has been amended to incorporate generic terminology for the trademarks present in the specification. Thus, it is believed that the objections to the trademarks set forth in the present specification have been obviated.

Claim 13-15, 17, and 33-38 were rejected under 35 U.S.C. § 102 (a) as allegedly being anticipated by ROWE et al. This rejection is respectfully traversed.

The claimed invention relates to an isolated polypeptide originating from a malaria crythrocyte membrane protein comprising an amino terminal part of the sequence

according to SEQ ID NO: 1. The claimed amino terminal portion is DBL-1, and the polypeptide is capable of binding to a negatively charged heparinase or heparinase-like molecule.

Applicants believe that the ROWE et al. publication fails to disclose or suggest the claimed invention. Applicants respectfully submit that the ROWE et al. references is directed to a polypeptide distinct from that of the claimed invention.

ROWE et al. teach on pages 293 - 294 that complement-receptor 1 (CR1) plays a role in rosetting. Moreover, ROWE et al. found that CR-1 deficient red blood cells showed reduced or no binding to the DBL-1 domain. On page 22, second paragraph, column 1, the article states that "The deletion of rosetting by heparinase treatment of normal RBCs gave us further information about this specific ligand-receptor interaction indeed suggesting that heparan sulfate, or a heparan sulfate-like molecule, is involved in the binding. Heparinase treatment also disrupted the rosettes of other strains of parasites (TM180, TM284), whereas the rosettes of the strain R29 were not affected (not shown)." Thus, contrary to the assertions of the Official Action, ROWE et al. teach a peptide distinct from the claimed invention.

In fact, the Examiners attention is directed to the enclosed sequence listing. It is believed that the sequence listing provides further evidence that ROWE et al. is directed a different polpeptide. The sequence listing shows the differences between the claimed DBL-1 domain and the R29 + clone polypeptide

as set forth in ROWE et al. Upon comparing the DBL-1 domain of P. *falciparum* parasites with the R29 clone, it is believed to be apparent that the amino acid sequences are distinct. The amino acids are aligned using the single amino acid code. Upon reviewing the sequence listing, it is evident that merely 32% of the amino acids are similar to each other. Thus, it is believed to be apparent that ROWE et al. fails to disclose or suggest the claimed invention.

Claims 24, 33, 34 and 38 were also rejected under 35 U.S.C. § 112, first paragraph for allegedly being based on a non-enabling disclosure. In light of the present amendment, this rejection is respectfully traversed.

Applicants respectfully submit that the present specification clearly enables one of ordinary skill in the art to make and use a virus containing distinct DBL-1 constructs of the SRCR3S1.2 VAR 1, in other words PfEMP1. Tests have shown that antibodies from immunized animals bind to the surface of red blood cells infected with P. *falciparum* and disrupted the preformed rosettes. Moreover, tests in vitro have shown that the adhesion of infected erythrocytes was hindered.

Applicants believe that due to constant human immune pressure, PfEMP1 molecules are variable in size and sequence. However, an increasing pool of data indicates that only a few species of PfEMP1 can cause severe episodes like cerebral or placental malaria. Interestingly, these PfEMP1s seem to be

commonly recognized by antibodies from individuals who are resistant to severe malaria. Thus, its likely that cerebral malaria or placental malaria can be prevented through immunizations with one or a few species of PfEMP1.

The N-terminal DBL-1 domain of PfEMP1 exists in all of PfEMP1 molecules. In fact, it is believed that this portion is the most conserved domain of the PfEMP1 domain. Two independent studies have confirmed that FCR3S1.2FR1/PfEMP1 is commonly recognized by antibodies from malaria immune serum (CARLSON et al., 1999, Chen et al., 2003).

In particularly, Applicants have discovered that immune-antibodies generated by a vaccination with recombinant PfEMP1-DBL-1 constructs of FCR3S1.2 recognize native PfEMP1 on a live infected red blood cell surface, disrupt preformed P. *falciparum* rosettes and hinder the adhesion of infected erythrocytes in an animal model (newly developed rat model, CHEN et al. 2003).

As mentioned in the specification on page 15, second paragraph, the ligand polypeptide of the invention preferably comprises 400 amino acids (DBL-1). Moreover, on page 39, it has been demonstrated that the DBL-1 region is involved in rosetting and that the addition of a DBL-1 fusion protein caused a dose-dependent rosette reversion.

As can be seen from Figure 2, the DBL-1 region is the most amino terminal portion of SEQ ID NO. 1. The potion is

comprised within a polypeptide having about 400-500 amino acids, the amino-terminal portion of the sequence according to SEQ ID NO. 1.

Accordingly, the claimed invention recites an isolated polypeptide originating from a malaria erythrocyte membrane protein comprising an amino part of the sequence according to SEQ ID NO. 1, wherein said amino terminal part is DBL-1 and wherein said polypeptide is capable of binding to a negatively charged heparan sulfate or heparan sulfate-like molecule. It is believed that the claimed invention is clearly enabled by the present disclosure.

Claims 13-15, 17, 21, 24 and 33-38 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

It is believed to be apparent that claims 13, 15, 17, 21, 24 and 33-38 have been amended so as to obviate the rejection that the claims are indefinite.

In the outstanding Official Action, claims 13-15, 24, and 33-38 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by HELMBY et al. This rejection is respectfully traversed.

HELMBY et al. disclose the isolation of small proteins, termed rosettings. They are surface antigens on P. *falciparum* infected red blood cells. However, proteins are not involved in rosetting, as demonstrated by FERNANDEZ et al. (J. Exp. Med., Vol. 190, 1999). A copy of this article is enclosed with this

amendment. On page 1394, first paragraph of column 1, polypeptides of low molecular mass are taught. These polypeptides have been found in rosetting strains, especially rosettings. Moreover, the polypeptides have a molecular size of less than 200 Kd. Applicants note that this is a size quite distinct from the known PfEMP1 antigens. Thus, it is respectfully submitted that HELMBY et al. fail to disclose or suggest the claimed invention.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application is now in condition for allowance, with claims 13, 15, 17, 21 and 33-38, as presented. Allowance to passage to issue on that basis are accordingly respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

YOUNG & THOMPSON

By Philip DuBois

Philip DuBois
Agent for Applicants
Registration No. 17,355
745 South 23rd Street
Arlington, VA 22202
Telephone: 521-2297

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Page 31, the paragraph, beginning on line 13, bridging pages 31 and 32, has been amended as follows:

--Two degenerate primers (DBL-1.1, 5'-GG(A/T) GC(A/T) TG(TC) GC(A/T) CC(A/T) T(A/T)(T/C) (A/C) G-3' (SEQ ID NO: 2); DBL-1.2, 5' -A(A/G)(A/G)T A(T/C)TG (T/A)GG (A/T)AC (A/G)TA (A/G)TC-3' (SEQ ID NO: 3) which mapped to the conserved region of all PfEMP1 DBL-1 were modified from the sequences of Su *et al.* The amplification parameters were first optimised so that the amplified products were visible with normal ethidium bromide (EB) staining (Cobb, B. D. and J.M. Clarkson, 1994. A simple procedure for optimising the polymerase chain reaction (PCR) using modified Taguchi methods, *Nucl. Acid. Res.* 22, 3801-3805). Briefly, one to five parasites, obtained by limiting dilution, were directly emerged in the RT-PCR buffer (Stratagene) with different concentration of primers, $MgCl^2$ KCl and Tris-Cl. Both DNA and RNA were released from the parasites by heating at 93°C for 3 min. The DNA was degraded by addition of 10 U DNase (Stratagene). Reverse transcription was carried out immediately after the addition of random primers and reverse transcriptase (Perkin-Elmer). The PCR reaction was subsequently performed in the same tube. Through comparison of the amplification efficiency from different reactions, the optimised parameters for single cell RT-PCR were found to be as follows: 100mM Tris-Cl,

pH 8.3, 35 mM $MgCl_2$, 500mM KCl, and the final concentration of primers was 1μM. In the subsequent experiments, individual trophozoite-infected rosetting erythrocytes were isolated with a 5μM glass-pipette using an inverted microscope. The selected pRBC was stripped of uninfected RBC and repeatedly grabbed, ejected and turned to conclusively ensure that it had pigment and that the selected cell was a single trophozoite-infected RBC (see Fig. 1A). Fifty cycles of amplification at 93°C for 20 seconds, 55°C for 30 seconds and 72°C for 1 min were needed for product detection. Several controls were included in each experiment; one blank control (without parasite(s)) and one without reverse-transcriptase to rule out the possibility of contamination and amplification due to the presence of genomic DNA.--

Page 32, the paragraph, beginning on line 24, bridging pages 32 and 33, has been amended as follows:

--A specific upstream primer (L-6, 5'-GAC ATG CAG CAA GGA GCT TGA TAA -3') (SEQ ID NO: 4) in the 434-bp sequence and a downstream primer (L-5, 5'-CCA TCT CTT CAT ATT CAC TTT CTG A -3') (SEQ ID NO: 5) mapping to the conserved sequence of ATS were generated and reverse transcription was carried out as described above. PCR was performed with the Expand™ High Fidelity PCR System (Boehringer Mannheim). A single 4.9-kb fragment was amplified, which was digested into three fragments with Hind III and EcoR V and cloned into the pZErO-1 vector (Zero Background, Invitrogen). The sequencing was performed with LongRanger™ gel

(FMC) on an A.L.F. Sequencer (Pharmacia). The 5′ region of the FCR3S1.2-*var*1 transcript was cloned by screening a cDNA library (Schlichtherle, unpublished) with the 434-bp fragment as probe and seven overlapping fragments were sequenced. The 3′ terminal region was cloned by nested RT-PCR. Reverse transcription was primed with oligo-dT and PCR was performed with a specific 5′ primer (P-1, 5′-CTT TCG ACT CTA CCA TCC T-3′)(SEQ ID NO: 6) upstream of TM region and a 3′ primer (P-4, 5′-TTA GAT ATT CCA TAT ATC TGA TA-3′) (SEQ ID NO: 7) mapping to the C-terminal sequence of FCR3 (*var* 2) PfEMP 1. Five overlapping fragments were sequenced. Fourteen overlapping clones were in total sequenced in both directions in order to ensure that the sequence was correct and was transcribed from a single gene.--

Page 34, the paragraph, beginning on line 1, has been amended as follows:

--Both DBL-1 and ATS fragments were amplified by specific primers (Ex-1.1, 5′-ATC GAA TTC TGC AAA AAA GAT GGA AAA GGA A-3′ (SEQ ID NO: 8) and D-1, 5′-GTA TTT TTT TTG TTT GTC AAA TTG-3′ (SEQ ID NO: 9) for DBL-1; Ex-2, 5′ - ATC GAA TTC TCT GAA AAT TTA TTC CAA A-3′ (SEQ ID NO: 10) and P-4 for ATS). The amplified fragments were inserted into the EcoR I cloning site of pGEX-4T-1 downstream of the glutathione S-transferase sequence. The *E.coli* BL21 was used as the expression strain. Expression of both fusion proteins was induced with 0.1 mM IPTG at 30°C for 4h and the fusion proteins were purified on glutathione sepharose

(Pharmacia) as described in the instructions provided by the manufacturer (GST Gene Fusion System, Pharmacia). The expression constructs were sequenced by cycle sequencing to check that the recombinant plasmids were of the expected sequences in the correct reading frames. Thrombin cleavage of the fusion proteins was performed according to a standard procedure. Western-blot analysis of DBL-1-GST and ATS-GST fusion proteins was with a biotin labelled anti-GST mAb (clone GST-2, IgG2b, Sigma) and ALP-avidin (Sigma) to reveal the pattern of protein expression. Although the induction of expression was at a low temperature and the purification was in the presence of a cocktail of enzyme inhibitors (0.5mM EDTA, 1mM [Pefabloc®SC] PEFABLOC (serine protease inhibitor) (AEBSF) Boehringer Mannheim), there was still some breakdown of the DBL-1-GST. The fusion proteins, stained by the anti-GST mAb, decreased with thrombin treatment. This information together with the knowledge that the plasmids were of the expected sequences ensured that the fusion proteins indeed were the corrected ones.--

Page 35, the paragraph, beginning on line 9, has been amended as follows:

--Ten-well immunofluorescence glass-slides were precoated with 10% poly-L-lysine in PBS for 30 min. Monolayers of RBC were made by addition of 20 µl of 0.5% 3x washed bloodgroup O Rh^+RBC in PBS to each well. Twenty µl DBL-1-GST, ATS-GST or GST alone (80µg/ml) in PBS was added to the wells for

30 min. The DBL-1-GST fusion protein was in subsequent experiments incubated in the presence of heparin, heparan sulfate or chondroitin sulfate (titrated from 20 to 8 mg/ml) to study the inhibitory activity of each GAG. Slides were washed 3 times with PBS and the fusion protein-binding was detected with the biotin-labelled anti-GST mAb and an [ExtraAvidin®] EXTRAVIDIN (a modified avidin) FITC conjugate (Sigma). The fluorescence was assessed in a Nikon Optiphot-2 UV microscope, using a x10 ocular and an oil lens with a magnification of x100.--

IN THE CLAIMS:

Claim 13 has been amended as follows:

--13. (thrice amended) An isolated polypeptide originating from a malaria erythrocyte membrane protein comprising an amino-terminal part of the sequence according to SEQ ID NO:1, wherein said amino terminal part is domain DBL-1, and wherein said polypeptide is capable of binding to a negatively charged heparan sulfate or heparan sulfate-like molecule.--

Claim 14 has been amended as follows:

--14. (twice amended) A polypeptide originating from a malaria erythrocyte membrane protein comprising [at least 300 amino acids of the sequence according to SEQ ID NO:1] domain DBL-1 having about 400-500 amino acids of the sequence according to SEQ ID NO:1.--

Claim 33 has been amended as follows:

--33. (twice amended) A medicament made from a polypeptide originating from a malaria erythrocyte membrane protein comprising an amino-terminal part of the sequence according to SEQ ID NO:1, wherein said amino terminal part is domain DBL-1, and wherein said polypeptide is capable of binding to a negatively charged heparan sulfate or heparan sulfate-like molecule.--

Claim 35 has been amended as follows:

--35. (twice amended) An isolated polypeptide originating from a malaria erythrocyte membrane protein comprising an amino-terminal part of the sequence according to SEQ ID NO:1, wherein said amino terminal part is domain DBL-1, and wherein the polypeptides bind to malaria-infected erythrocytes membrane protein, and wherein said polypeptide is capable of binding to a negatively charged heparan sulfate or heparan sulfate-like molecule.--

Claim 38 has been amended as follows:

--38. (amended) A composition comprising and adjuvant and a polypeptide according to claim 13 [in a vaccine].--